A Comparison of RIA and BACI Analysis for Detecting Pollution Effects on Stream Benthic Algal Communities

Susan L. Eggert
Department of Fisheries and Wildlife
Michigan State University
East Lansing, MI 48824

Thomas M. Burton
Departments of Fisheries and Wildlife and Zoology
Michigan State University
East Lansing, MI 48824

Dennis M. Mullen
Department of Zoology
Michigan State University
East Lansing, MI 48824

Abstract
Before and After, Control and Impact analysis (BACI) and Randomized Intervention Analysis (RIA) are used to overcome pseudoreplication in sampling designs that emphasize data collection at paired control and impacted sites. Both methods were applied to data collected on the potential effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation on benthic diatom communities at two sites in the Ford River, Michigan. Data on the diatoms were collected as part of the U.S. Navy funded studies (through IIT Research Institute) on ELF effects from an ELF communication antenna. Data on chlorophyll a, diatom species diversity, and diatom abundance were collected at an impact site under the antenna and at a control site receiving 7-10 times less ELF radiation than the impact site. Data were divided into a 3 year before period and a 6 year after period. Both procedures detected differences ($p < 0.05$) in the relationship between the two sites in chlorophyll a and species diversity before antenna operation as compared to the period after antenna operation began, but detected no difference in diatom abundance. The RIA procedure was limited by sample sizes less than 40, limiting our ability to detect monthly differences with it. The small sample size problem emphasizes the need for long term monitoring. The parametric (BACI) and randomization (RIA) procedures offer powerful, complimentary tools for the detection of pollution effects using control and impact sites.

Key Words: Statistical applications, BACI analysis, RIA analysis, diatoms, rivers, ELF electromagnetic radiation.

Introduction

The experimental design of large-scale monitoring efforts used to determine environmental effects of a pollutant has commonly been plagued by the problem of psuedoreplication as defined by Hulbert (1984). A typical sampling design, set up to determine whether a known pollutant will adversely affect a biological parameter, consists of replicated sampling over time at a control and impact site. However, funding and logistic constraints associated with environmental monitoring often makes the replication of treatments (sites) impossible. This lack of replication, therefore, invalidates the use of inferential statistics such as analysis of variance in interpreting the data (Stewart-Oaten 1986).

The problem of statistically detecting non-random changes between a single control and impacted site has received some attention in the literature (Stewart-Oaten et al. 1986, Carpenter et al. 1989, Carpenter 1990, Jassby and Powell 1990, Reckhow 1990).

Stewart-Oaten et al. (1986) have introduced the Before and After, Control and Impact analysis (BACI), which requires paired sampling at control and impact sites, both before and after perturbation. The mean of the "before" differences between sites is compared to the mean of the "after" differences between sites by a t-test. If the magnitude of the difference between sites changes significantly following addition of the pollutant, there may be a perturbation effect. The procedure assumes that the following criteria are met: (1) the measures of the parameters at any time are independent of the measures at any other time, and (2) the differences between control and impact sites of the "before" period are additive.

Randomized Intervention Analysis (RIA) represents a non-parametric alternative to the BACI analysis (Carpenter et al. 1989). RIA is based on replicated sampling over time, before and after an impact, at control and experimental sites. A mean difference between sites is calculated from both "before" and "after" data sets. The absolute value of the difference between these means represents the test statistic. Random permutations of the time series of inter-site differences provide an estimate of the distribution of the test statistic. The proportion of randomly created differences between means that are greater than the observed difference between means, determines whether a significant change has occurred between sites. By using a randomly created error distribution, the RIA design does not require transformations for non-additive data. RIA does require the assumption that errors are independent from one another through time. However, Carpenter et al. (1989) indicated that serial correlation did not lead to ambiguous results in 97 percent of the autocorrelated cases that they examined.

The objective of this paper is to demonstrate the potential use of RIA and BACI as statistical tools for the detection of pollution effects in environmental monitoring. Here we compare and contrast both methods using diatom abundance, species diversity and chlorophyll a standing stock data sets collected as part of an on-going study to monitor potential effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation on stream benthic diatom communities at a control and impact site in the Ford River, Michigan.

Methods and Materials

The data were obtained from an ongoing study of the effects of ELF electromagnetic radiation (generated by the U.S. Navy's ELF antenna) on the Ford River ecosystem in Michigan's upper peninsula (see Burton et. al. in this volume for more details on this study). Glass microscope slides held in plexiglass carriers and incubated in the river for 28 days (42 days during the winters of the last three years of the study) were used to sample the periphyton community at two sites in the river. The impact site lies directly beneath the antenna and receives about 7 times the exposure of the control site, which is about 8 km downstream from the antenna. Pre-operational data were collected every 28 days between June 1983 and May 1986. Operational data were collected every 28 days during the spring, summer and fall and every 48 days during the winter between June 1986 and September 1990. Testing on the antenna began in May 1986 at 4 amps (impact site exposure rate = 1.6 mv/min). On April 28, 1987 the power was increased to 15 amps (6.1 mv/min at the impact site) and on November 15, 1987 the power was increased to 75 amps (31.0 mv/min at the impact site). Testing at full power (150 amps, 61.0 mv/min at the impact site) began on May 1, 1989 and full operations at 150 amps began on October 7, 1989. The antenna was operated in a fairly irregular pattern during the testing period from May 1986 to October 1989.

BACI analysis and RIA were conducted on chlorophyll *a* standing crop (10 replicates per site per sampling date), species diversity (3 replicates per site per sampling date), and individual diatom species abundances (3 replicates per site per sampling date). Each data set was split up into "before" and "after" periods with all sampling dates from June 1983 to April 1986 as the "before" period and all dates from May 1986 to September 1990 as the "after" period. The data for the biological parameters and diatom abundances were also divided into summer and winter seasons to statistically examine seasonal variations for these parameters. Seasons consisted of a Summer (May to October) and a Winter (November to April) period. Those seasons prior to Summer 1986 represented the "before" period, while the "after" period consisted of all seasons after May 1986. Using the BACI technique, we also compared individual seasons of the "before" period to other "before" seasons to determine whether any differences occurred prior to impact. Each of the "before" seasons was then compared to each of the "after" seasons to see whether significant differences between sites had occurred. Finally, peak diatom abundances were compared between sites using BACI for those months of the year when species such as Achnanthes minutissima become dominant.

The BACI analyses were run as specified by Stewart-Oaten (1986). Data points from each sampling date were entered into files using Statview 512+ on a Macintosh Plus microcomputer. The set of "before" data were tested for additivity using Tukey's one degree of freedom test for non-additivity. If the slope of the regression of differences between sites against the means of both sites varied significantly from zero ($p < 0.05$), then data were transformed. The log (x + 1) transformation was used for non-additive biological data, while the arcsin square root of the mean transformation suggested by Steel and Torrie (1960) was used for proportional data. According to Stewart-Oaten et al. (1986) the independence of error assumption required by the BACI analysis may be considered to be "plausible if large, local, long-lasting random effects are unlikely". While our initial analysis of the data indicated that this assumption was indeed plausible, any significant or questionable results were closely examined for possible serial correlation problems. The Durbin-Watson test (1951) was used to test for independence of errors. If the "before" data sets met the additivity and independence of error criteria, differences between sites for each time period were compared with an unpaired two-tailed t-test. Data sets which failed to meet the stringent requirements of BACI analysis were analyzed using the non-parametric RIA test.

Data for RIA were entered into data files using the Supercalc software program and transferred to separate ASCII files for each parameter for each site. By using a randomly created error distribution, RIA eliminates the criterion for additive data. RIA calculations were performed on an IBM microcomputer using the RIAPUB program obtained from Dr. Stephen R. Carpenter. The program is interactive in nature and is applicable for most studies of this type. A probability distribution created by RIAPUB through random permutations of the time-series of inter-site differences determined whether a significant change between sites occurred after antenna operation.

Results and Discussion

Pooled and seasonal chlorophyll a standing crop data for the "before" period were found to be non-additive using Tukey's test. A plot of mean standing crop against differences between sites using raw data showed the slope to be significantly greater than zero (Figure 1a and 1c). The log (x + 1) transformed data set was retested for additivity and the slope of the regression line was found to be not significantly different from zero (Figure 1b and 1d). Results of a BACI unpaired t-test on pooled chlorophyll a data indicated a significant difference ($p < 0.01$) between "before" and "after" period mean differences (Table 1). When the data

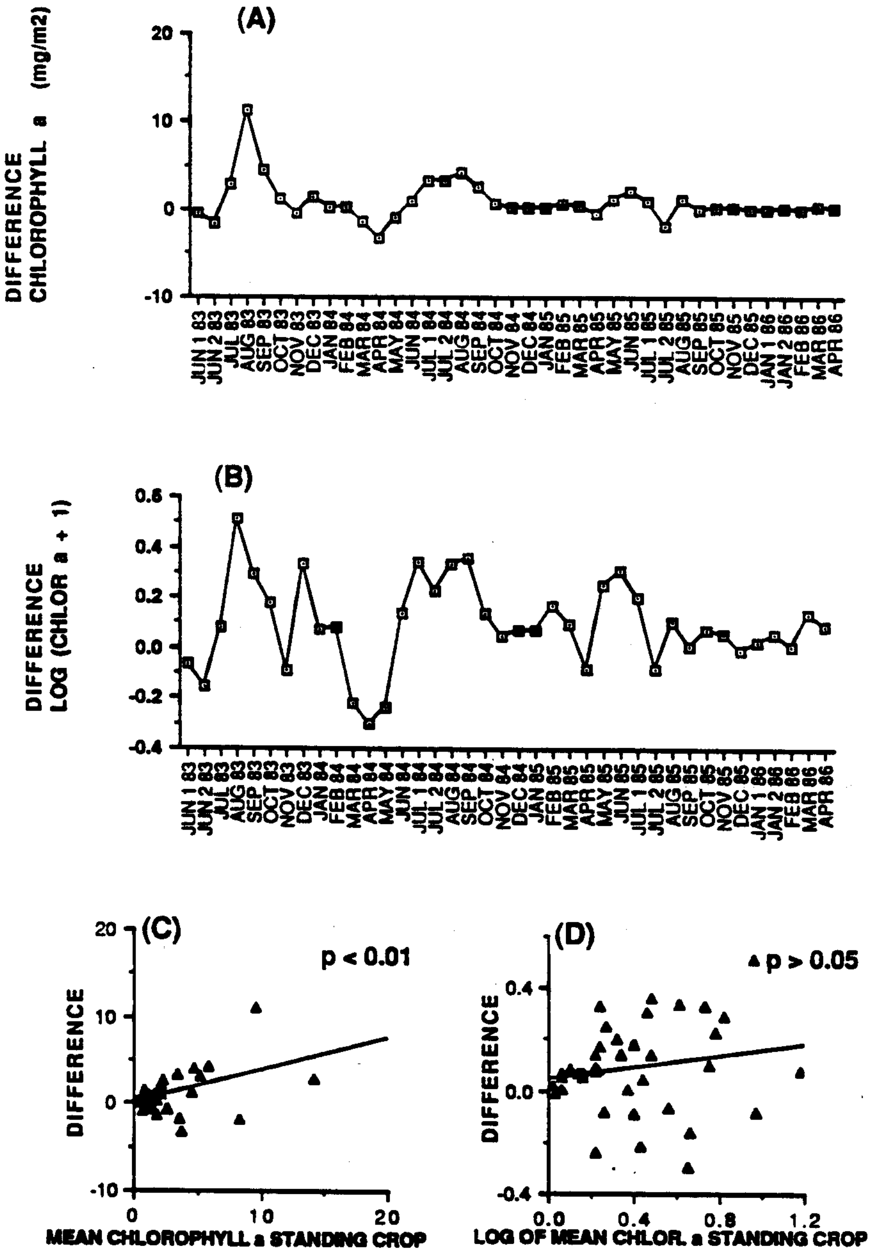

Figure 1. Non-additive and additive chlorophyll a data. (A) Difference between sites of raw chlorophyll a data for "before" period. (B) Difference between sites of log(x+1) transformed chlorophyll a data for "before" period. (C) Tukey's test for non-additivity on raw chlorophyll a data (significant at $p < 0.05$). (D) Tukey's test for non-additivity on log transformed chlorophyll a data (non significant).

Table 1. Summary of BACI and RIA comparisons for chlorophyll a, species diversity and diatom abundance between control (FCD) and experimental (FEX) sites for 1983-1990. N in parentheses for BACI and RAI, respectively.

Parameter	Comparison	BACI Signif. ($p < 0.05$)	RIA Signif. ($p < 0.05$)
Chlorophyll a	6/83-4/86 vs. 5/86-9/90 (84) (84)	$p < 0.01$	$p < 0.05$
	Summer 83-85 vs. 86-90 (49) (51)	$p = 0.06$	$p < 0.01$
	S 83/88 (9)	$p < 0.05$	
	S 83/90 (9)	$p < 0.05$	
	S 84/87 (11)	$p < 0.05$	
	S 84/88 (10)	$p < 0.05$	
	S 84/90 (10)	$p < 0.01$	
	S 85/87 (11)	$p < 0.05$	
	S 85/88 (10)	$p < 0.01$	
	S 85/90 (10)	$p < 0.01$	
	Winter 83-85 vs. 86-89 (33) (33)	NS	NS
Species Diversity	6/83-4/86 vs. 5/86-9/90 (83) (85)	$p < 0.05$	$p < 0.05$
	Summer 83-85 vs. 86-90 (48) (51)	NS	NS
	Winter 83-85 vs. 86-89 (33) (34)	NS	NS
Diatom Abundance A. minutissima			
	Summer 83-85 vs. 86-90 (45) (46)	NS	NS
	Winter 83-85 vs. 86-89 (31) (33)	NS	NS
	May & June 83-85 vs. May & June 86-90 (12)	NS	--

were broken down on a seasonal basis, significant differences between the summers of 83, 84 and 85, and the summers of 87, 88 and 90 were found. A closer inspection of the chlorophyll a "before" data set revealed that the independence assumption was not completely satisfied. Results of a Durbin-Watson test on "before" period data indicated that a significant ($d = 1.14$, $p < 0.05$) serial correlation problem existed.

Chlorophyll a standing crop data were then analyzed using the non-parametric randomized intervention analysis. The inter-site relationship changed over time for both the entire data set and the summer seasonal data (Table 1). Due to the lack of sensitivity of RIA at observation numbers less than 40, year-to-year comparisons could not be run (Carpenter et al. 1989).

Diatom species diversity data were found to be additive using Tukey's test for pooled ($p <$

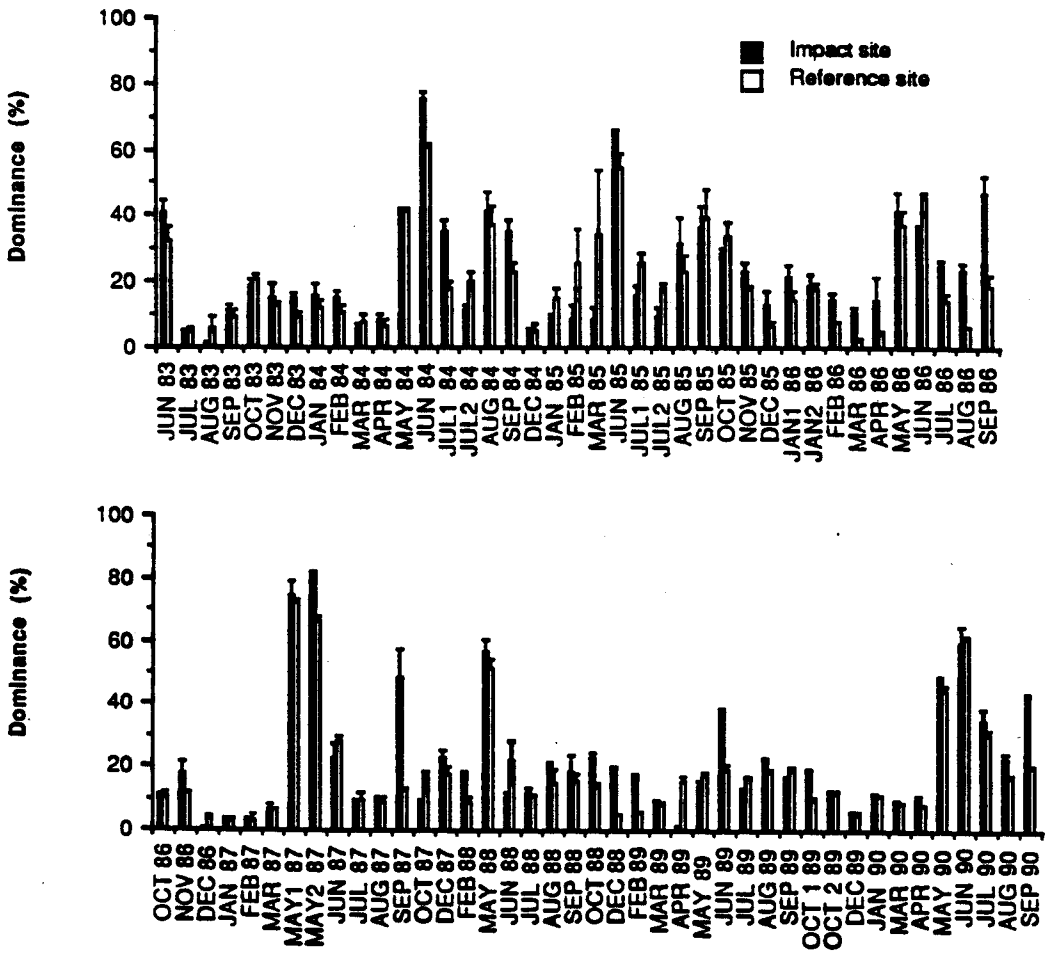

Figure 2. Achnathes minutissima percent dominance for the Ford River, 1983-1990.

0.84) data sets, and summer ($p < 0.90$) and winter ($p < 0.96$) data sets. Results of Durbin-Watson's test indicated no serial correlation problem ($d = 1.60$, non-significant at $p < 0.05$). Since all data sets for diversity passed BACI's additivity and independence tests, an unpaired t-test was run on untransformed data for pooled, seasonal and year-to-year comparisons. Results of BACI t-tests demonstrated significant changes ($p < 0.05$) in the inter-site relationship for the pooled"before" and "after" data (Table 1). Seasonal pooled comparisons were not significant. The only year-to-year comparison that was significant was the summer 1983 to summer 1987 comparison.

Species diversity pooled and seasonal data analyzed using RIA reflected the results of the BACI analysis (Table 1). In this instance, additional statistical analysis of diversity data using the non-parametric randomized intervention analysis was redundant. A check of all data sets indicated that all assumptions of BACI had been satisfied, thus making RIA unnecessary.

The abundance of a dominant diatom species Achnanthes minutissima has followed a predictable pattern of high dominance during summer periods and low dominance during the winter (Figure 2). All diatom abundance data were transformed using the arcsin square root of the mean transformation and tested for additivity. Seasonal transformed data for the before period were marginally additive (summer: $p < 0.06$ and winter: $p < 0.08$). Durbin-Watson independence tests of summer data found no significant autocorrelation problem ($d = 1.98$), while winter data were significantly autocorrelated ($d = 0.90$, $p < 0.05$). Results of unpaired t-tests for A. minutissima indicated that there were no significant inter-site changes in mean differences for either seasonal "before" and "after" periods, or year-to-year comparisons. Since the winter abundance data were found to be significantly autocorrelated, BACI results were verified using RIA. RIA reflected the results obtained in the BACI comparisons of both the summer and winter abundance data (Table 1).

In an attempt to detect even more subtle changes in diatom abundances, we ran BACI analyses on monthly data at peak A. minutissima abundances (Figure 2). All May and June data for the years 1983-1985 were pooled to represent the "before" period and all May and June data for 1986-1990 as the "after" period so that mean differences beween sites could be examined. The data appeared to be significantly negatively serial correlated ($d = 3.33$, $p < 0.05$). Since negative autocorrelations are conservative with regard to probability levels, an unpaired t-test was run on the monthly data. There was no significant change in the inter-site relationship after antenna operation according to the BACI analysis (Table 1). This comparison could not be verified with RIA due to the limited number of observations available.

For the chlorophyll a standing crop and species diversity parameters where significant differences were found using either BACI or RIA, the data were scrutinized further to determine whether ELF electromagnetic radiation or another factor had caused the observed differences. Significant differences found by BACI or RIA do not imply that a suspected perturbation has caused a change, nor do these tests reveal at what point in time the change occurred. Ecological and procedural considerations should be examined in all cases. Analysis of covariance (ANCOVA) of chlorophyll a standing crop with ELF exposure included as a covariant indicated that a variable other than ELF electromagnetic radiation caused the change in relationship between sites. Significant positive correlations between water temperature and chlorophyll a during drought periods from 1986 to 1990 suggest that the observed differences were related to weather variables. ANCOVA of species diversity data using ELF exposure as a covariate also indicated that a factor other than antenna exposure was responsible for the significant BACI and RIA results.

Along with the need for careful interpretations regarding the possible sources of variation, data set sample sizes should be considered when deciding on the appropriate statistical analysis. Generally, the statistical power of non-parametric tests such as RIA is smaller than that of a similar parametric test (Welkowitz et al. 1976). When populations are normally distributed, the number of observations required by RIA should be larger than the sample size required by the BACI analysis in order to obtain the same amount of power. Carpenter et al. (1989) reported that RIA could consistently detect manipulation effects with sample sizes of 40 or more. Stewert-Oaten et al. (1986) did not suggest a minimum sample size for the BACI analysis. Although the authors did present an example in which only 23 data points were used to detect a manipulation effect, Monte Carlo simulations are required to definitively determine the effective sample size required by the BACI method. Thus, year-to-year BACI comparisons presented in this study should be interpreted with some caution, since BACI's ability to detect perturbation effects with sample sizes of nine to eleven (Table 1) data points remains unknown. The sample size problem also emphasizes the need for long term monitoring of potential pollutant effects.

In summary, an environmental impact study should provide quantitative evidence to support regulatory decisions regarding potential environmental pollutants. Both the BACI analysis and RIA offer a means of quantitatively detecting whether perturbations such as toxic effluents, pipeline construction, or power plant discharges may be impacting an ecosystem. The BACI and RIA results should be interpreted with some care and caution however, as significant findings do not imply that the suspected pollutant has caused the observed differences. Our analysis of ELF effects on a riverine algal community using BACI and RIA suggests that the following statistical protocol will accurately and quantitatively allow the detection of environmental perturbations. First, the parametric BACI analysis should be used for data sets satisfying plausible assumptions of independence and additivity. If the relationship between control and impacted sites has changed significantly over time, or if the independence, normality or additivity assumptions appear to be questionable, then the non-parametric randomized intervention analysis may be used to examine the data. Finally, if the inter-site relationship is found to change over time using RIA, final conclusions of the perturbations effects should be based on ANCOVA results (using the magnitude of the perturbation as the covariate) and/or other ecological considerations. When used in this manner, BACI and RIA represent complimentary and practical tools with which to make sound ecological decisions regarding potential environmental impacts.

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Literature Cited

Burton, T. M., D. M. Mullen, and S. L. Eggert. 1991. Effects of extremely low frequency (ELF) electromagnetic fields on the diatom community of the Ford River, Michigan. pp. 17-25 In: T.P. Simon and W.S. Davis (editors). Proceedings of the 1991 Midwest Pollution Control Biologists Meeting. U.S. EPA Region V, Environmental Sciences Division, Chicago, IL. EPA-905/R-92/003.

Carpenter, S. R. 1990. Large-scale perturbations: opportunities for innovation. Ecology 71:2038-2043.

Carpenter, S. R., T. M. Frost, D. Heisey, and T. K. Kratz. 1989. Randomized intervention analysis and the interpretation of whole-ecosystem experiments. Ecology 70:1142-1152.

Durbin, J. and G. S. Watson. 1951. Testing for serial correlation in least squares regression II. Biometrika 38:159-178.

Hulbert, S. H. 1984. Psuedoreplication and the design of ecological field experiments. Ecological Monographs 54:187-211.

Jassby, A. D. and T. M. Powell. 1990. Detecting changes in ecological time series. Ecology 71:2044-2052.

Reckhow, K. H. 1990. Bayesian inference in non-replicated ecological studies. Ecology 71:2053-2059.

Stewart-Oaten, A., W. W. Murdoch, and K. R. Parker. 1986. Environmental impact assessment: "Pseudoreplication" in time? Ecology 67:929-940.

Steel, R. G. and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York, 481 pp.

Tukey, J. W. 1949. One degree of freedom for non-additivity.Biometrics 5:232-242.

Welkowitz, J., R. B. Ewen and J. Cohen. 1976. Introductory statistics of the behavioral sciences. 2nd edition. Academic Press, New York, 316 pp.

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Region 5
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